The University of Chicago

THE EFFECT OF CARBOHYDRATES AND RELATED COMPOUNDS ON THE ISOLATED RABBIT GUT

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
AND PHARMACOLOGY
1934

By
BERT JOHN VOS, Jr.

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
TECHNIQUE AND APPARATUS	3
EXPERIMENTAL RESULTS	7
The Gut From the Unstarved Rabbit	
The Gut From the Starved Rabbit	
Activity in Glucose-Containing Solutions	
Activity in Glucose-Free Solutions	
Effect of Substances Other Than Glucose	
SUMMARY	24
REFERENCES	25

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INTRODUCTION

The first recognition of the importance of an adequate supply of carbohydrate in physiological salt solutions was made by Locke (1) in his work on the isolated frog heart. These studies were extended by him (2) to the isolated mammalian heart and here he was able to show that hearts which ordinarily became quiescent after 1/2 - 3/4 of an hour would continue to beat for seven or eight hours if glucose in a concentration of 0.1 per cent were added to the solution. Sucrose, maltose, and lactose had no such effect, but fructose seemed to have a slight effect. Later Locke and Rosenheim (3) added galactose, rhamnose, l-arabinose, and glucoheptose to the list of substances which have no action on the isolated heart comparable with that of glucose.

Cognizant of Lock's work Magnus (4) included the question of sugars in his studies on the isolated gut. In experiments on the intestines, chiefly from well nourished cats and rabbits, he was unable to show any beneficial effects from the addition of glucose or sucrose in various concentrations to the physiological salt solution in which the gut was bathed. Although he was able in one instance to add sucrose to the bath in a concentration of one per cent without disturbing the normal motility of the gut, in most cases the addition of sugar even in as small quantities as 0.02 per cent was followed by weakness and irregularity of the movements and a disappearance of the spontaneous tone changes.

In their studies on intestines taken from previously starved rabbits Neukirch and Rona (5, 6, 7) had no difficulty in showing the importance of glucose in maintaining the contractions or in restoring the contractions after they had dwindled in the absence of glucose. In examining nearly seventy different chemical compounds, which included carbohydrates, alcohols, fatty acids, and their derivatives, only the following were found to have a stimulant action on the gut: glucose, mannose, galactose, sodium acetate, sodium butyrate, inactive sodium beta hydroxy butyrate, sodium pyruvate, sodium oxalacetate, and optically inactive sodium lactate. Of these mannose and sodium pyruvate had an action equal to that of glucose and the remaining six were only weakly stimulating. Le Heux (8) felt that the activity of the acetate and pyruvate might be a consequence of their esterification with the choline normally present in the gut and the formation of acetyl and pyruvyl choline, both of which are many times more stimulating

to the gut than choline itself. Jendrassik and Tangl (9), however, showed that this explanation was not valid since sodium pyruvate and acetate are still active after atropinization of the gut, whereas the corresponding choline esters are then without effect.

The present problem was directed at obtaining information of a more quantitative character than that reported by previous workers concerning the relative activities of the various carbohydrates, and compounds related to them, on the isolated rabbit gut. Since glucose was selected as the standard to which the activities of all other substances would be referred, it was first necessary to study its action in as much detail as possible.

TECHNIQUE AND APPARATUS

In as much as the present studies were directed at the effect of carbohydrates on the isolated rabbit gut, it seemed desirable to reduce the naturally occurring store of energy and thus render the gut more dependent upon an external source. To this end the rabbits were starved for thirty-six hours with a liberal supply of drinking water in cages with false bottoms to prevent them from having access to their feces. The animals were killed by a blow on the head and portions of the small intestine were removed to an evaporating dish containing oxygenated physiological salt solution. Here the mesentery was trimmed off and the gut was flushed out with additional physiological salt solution at room temperature. One segment was then used immediately and the others were stored in oxygenated, glucose-free physiological salt solution in the refrigerator at 4° C. No preparation was used more than fourteen hours after the death of the animal. A record was kept of the level of the intestine from which the various portions were taken.

These gut preparations, which measured about 3 cm. in length, were tied shut at both ends and the contractions of the longitudinal muscular coat were recorded by the Magnus suspension method on the smoked drum of a kymograph. In the majority of the experiments the behavior of only one strip of gut was studied at a time. The bath of solution in which the gut was immersed had a volume of 15 cc. This bath was in turn kept in a three liter beaker which was filled with water and maintained at 38° C. During the course of the experiment fresh physiological salt solution was passed at a constant rate of 10 cc. per minute from a stock reservoir through a warming coil immersed in the larger bath and then into the inner bath. As the fresh solution entered the bath it was rapidly mixed with the solution already present by a stream of five per cent CO_2 in oxygen. The old solution was removed through a side arm attached to the bottom of the bath. A constant level device kept the volume of solution plus gut at 15 cc. Since the volume of the gut was usually 2 - 2.5 cc., approximately 13 cc. of solution was in immediate contact with the gut at one time. This process of passing a steady stream of fresh solution through the bath will hereafter be referred to as "circumfusion."

Analyses of the bath contents when the solution was flowing through at the rate of 10 cc. per minute showed the

completeness of change from one solution to a new one to be as given in Table I. The initial lag is a consequence of the time (approximately one minute) needed for the solution to pass through the warming coil.

TABLE I

Time In Minutes	Amount of Change in Per Cent
2	26
4	83
6	98

This somewhat laborious process of circumfusion was adopted to meet a twofold need. In the first place it was noticed in some of the preliminary experiments that when a gut had been contracting for a long period of time in a standing solution, the replacement of this by a fresh quantity of the same solution was frequently attended by an improvement in the tone and amplitude of the gut's contractions. Since this phenomenon occurred in glucose-free solutions it was interpreted as being due to an accumulation of non-volatile metabolites in the bath rather than to an exhaustion of any of the active constituents of the solution. It was apparent that if one wished to compare the effects of two stock solutions containing different carbohydrates it would be necessary to control in some manner the changes produced by merely renewing the solution. The simplest way of controlling this seemed to be to renew the solution constantly. Thus one circumfuses the gut with a physiological solution containing 5.0 mM. of glucose for an hour and then without stopping the flow, changes to a solution containing 7.0 mM. of mannose. In the second place circumfusion of the gut with a solution containing no glucose offers an excellent opportunity to compare in a quantitative manner the brief effects of a variety of substances. They were added in isotonic solution directly to the bath by means of a pipette. If they are active the gut shows a stimulation which passes off as the unused portion of the substance is washed out of the bath by the circumfusing solution. By alternating doses of the substance under investigation with doses of glucose and comparing the effects which the two produce on the gut it is possible to conduct a bioassay and arrive at quantitative information regarding the relative activity of the two substances. By observing the proper precautions (submaximal responses, uniformly spaced dosages, and steady

rate of circumfusion) it is possible to detect difference as low as fifteen to twenty per cent.

In a few experiments two strips of gut which had been taken from immediately adjacent portions of the intestine but which had received different preliminary treatment, were used. These were then placed in the same bath and the movements of each were recorded one above the other on the same kymograph drum.

In still other experiments two strips of gut from adjacent portions of the intestine which had received identical preliminary treatment were allowed to contract in separate baths and were circumfused simultaneously with different physiological solutions. Thus a glucose-free solution might be passed through one bath and a glucose-containing one through the other.

The stock physiological salt solution used in all of these experiments resembles quite closely the one used by Van Dyke and Hastings (10) in their studies on the isolated guinea pig uterus. Its composition is given in Table II.

TABLE II

Constituents	Concentration	
	Millimols	Per Cent
NaCl	121.8	0.71
$NaHCO_3$	23.1	0.19
KCl	4.0	0.30
$MgCl_2$	1.0	0.0095
$CaCl_2$	0.9	0.010
Na_2HPO_4	0.4	0.0058
KH_2PO_4	0.1	0.0014
Phenol Red		0.00075

The essential difference between this solution and ones used by earlier workers lies in its higher bicarbonate ion concentration and in the fact that it is kept in equilibrium with five per cent CO_2 in oxygen. As a consequence of this latter feature the solution has a pH of 7.4 which remains constant throughout the experiment instead of starting at 7.9 and rising further as is the case with Tyrode's and similar solutions where the aeration with pure oxygen gradually washes CO_2 out of the solution. The phenol red served as an indicator in making up the solution, the calcium and magnesium chlorides being added after

the solution had been rendered acid by an excess of CO_2. After the addition of the calcium and magesium chlorides the solution was shaken with oxygen until the pH was approximately correct and then finally equilibrated with five per cent CO_2 in oxygen.

Control experiments showed that phenol red in the concentration used was without effect on the motility of the gut.

When glucose or any other carbohydrate was added to the stock solution it was first made into a solution that had an equal osmotic pressure (0.318 M.) and then an appropriate amount of this solution was used in place of an equal amount of isosmotic sodium chloride. In this way the osmotic pressure was left undisturbed and changes in concentration involved only the sodium and chloride ions. When the carbohydrate was added directly to the bath in which the gut was contracting, isosmotic solutions were again employed, but here, of course, all of the ions in the bath were diluted. With small amounts of inert carbohydrates this was without effect on the tracing made by the gut. When the quantity of inert solution added amounted to ten per cent or so of the bath volume there were frequently disturbances in the tracings. For reasons which will be explained in the discussion of the action of galactose these effects were interpreted as being due to a dilution of active ions rather than to a weak activity of the substance added.

EXPERIMENTAL RESULTS

The Gut From the Unstarved Rabbit

When a gut taken from a normal unstarved rabbit is placed in a glucose free physiological solution the results obtained are subject to considerable variation. Occasionally such a gut will contract well for seven or eight hours, but more often the contractions are markedly dimished before three hours have elapsed. It is possible that these variations are due to different nutritional states of the animals. Different gut preparations from the same animal usually show similar powers of endurance.

The Gut From the Starved Rabbit

Activity in Glucose-Containing Solutions

The solution most frequently used in these experiments contained 5.5 mM. of glucose, that is, 100 mgm. per 100 cc. Although this is somewhat in excess of the glucose occurring normally in the blood, it seemed a desirable concentration to employ both in view of the limited opportunities for absorption offered by the intestinal serosa in contrast to those of the capillary bed, and in view of the fact that this quantity of glucose has been accepted as standard for physiological solutions.

The behavior of a gut placed in such a solution within half an hour after the death of the animal is as follows: First there is a period, lasting for about twenty minutes, in which there are strong, even contractions of the longitudinal muscle. The tone falls steadily and the contractions gradually diminish in size and finally become quite irregular. During this first period the muscle in the circular coat appears to be quite inactive. In the second period, which lasts for one or two hours, the tone soon rises to its previous maximum, the amplitude of the contractions is recovered, but the irregularity in the amplitude of the contractions persists. In watching the gut one gets the impression that parts of the longitudinal muscle are contracting at varying intervals instead of there being a simultaneous contraction of the whole muscle. There are frequent contractions of the circular muscle with the formation of stationary constriction rings and peristaltic movements. A typical tracing showing these first two periods of activity is seen in Figure 1. At some time, usually

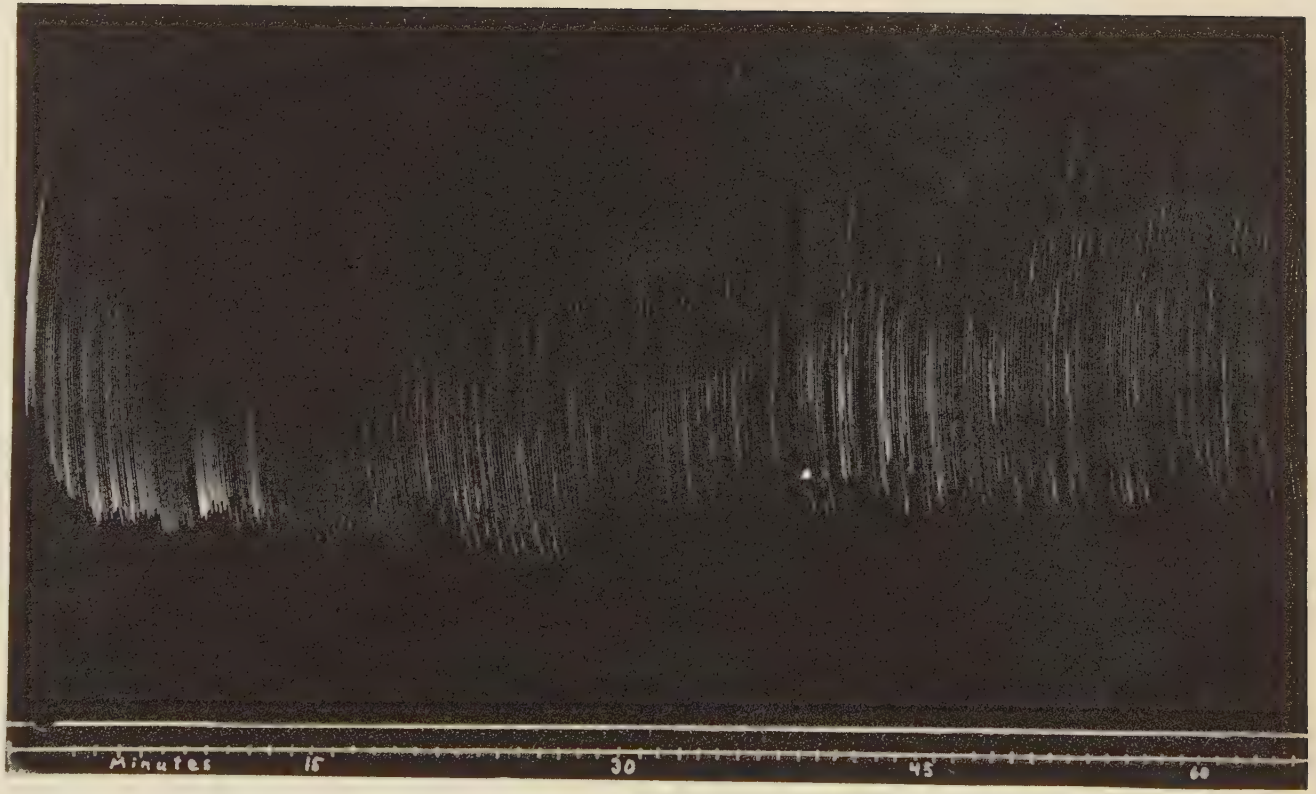

Fig. 1.- First and second phases of activity of an unstored gut in physiological solution containing 5.5 mM. of glucose.

two to three hours after the start of the experiment, the gut passes gradually into the third phase of its activity. The contractions become more and more regular and their amplitude increases by virtue of the fact that the whole longitudinal muscle is now contracting at the same time. From now on there is a long plateau in which the regularity is well maintained, the amplitude decreases only very gradually, the frequency of the contractions increases, and the tone rises slowly. In one gut the contractions were only reduced to 1/3 of the previous maximum and were still quite regular at the end of ten and one-half hours of activity. Usually, however, six or seven hours is the period during which a gut will maintain sufficient amplitude to make a study of the effects of drugs or ions profitable. Although the circular muscle is apparently quite quiescent during this third phase of the gut's activity, it is not completely asphyxiated, for it can still be aroused to a vigorous contraction by such drugs as physostigmine and pilocarpine.

The behavior of guts which have been stored in physiological solution in the refrigerator before use is as follows: If the gut has been stored for a long time, twelve hours or so, it may remain quiescent for ten minutes after being placed in the bath before it starts to contract. If the gut has been stored for a shorter period it is found to contract as soon as it is placed in the warm bath and submitted to the slight tension which the writing lever exerts on it. In either case the first contractions are small and infrequent. As time goes on these contractions gradually increase in amplitude and frequency. Coincident with this there is a fall in tone. In the case of the intestinal strips which have been stored for a long period this fall in tone takes place during the initial quiescence rather than during the first contractions. The second, or irregular phase of activity which is so characteristic of fresh preparations is much less conspicuous in stored ones. When the gut has been stored for only a short while, the appearance of this irregular phase is merely delayed and its duration shortened. With guts which have been stored seven to fourteen hours the irregular phase is almost uniformly absent. In these the third phase is merely a continuation of the first phase but it is a distinct phase in guts which have been stored only a short while. Here the contractions proceed with a beautiful regularity. As with fresh guts the amplitude of the contractions decreases only very slowly, and there is a gradual rise in tone. The gut usually contracts well for as long as six hours and occasionally up to nine and ten hours.

Several experiments were run in which a gut was circumfused alternately with two solutions having different glucose concentra-

tions. In these experiments no difference could be detected between the behavior of the gut in 4.0 mM. of glucose and 5.5 mM. In comparing 3.5 mM. with 5.5 mM. it was noted that upon changing to the latter concentration there was a slight but definite increase in tone and amplitude. When the glucose concentration was reduced from 5.5 mM. to 3.5 mM. the corresponding loss in tone and amplitude was much more gradual.

In frequent experiments the effects of an excess of glucose were tested by adding an isotonic glucose solution directly to the bath in which the gut was being circumfused by a solution which already contained the normal (5.5 mM.) amount of glucose. Even though the concentration of the glucose was in some cases raised to 18.4 mM. a response on the part of the gut was obtained in only one instance. In this case the gut had been previously exposed to a glucose-free solution for some time, then after a brief exposure to the 5.5 mM. glucose solution it was possible to elicit a slight response by elevating the concentration of the glucose in the bath to 16.9 mM. The response appeared as a slight increase in tone which disappeared as the excess glucose was washed out by the circumfusing solution.

Mannose, the action of which bears a striking resemblance to that of glucose, was likewise without effect in doses as high as 11 mM., when administered to a gut contracting in an adequate supply of glucose.

In addition to this, numerous other carbohydrates, which, as will be described later, had little or no effect on the gut in the absence of glucose, were likewise without effect in its presence. Among these were l-arabinose, xylose, galactose, fructose, sucrose, and maltose.

In contrast to these sodium d-lactate and sodium pyruvate were both able to stimulate the gut in the presence of 5.5 mM. of glucose. With sodium d-lactate the effects were elicited with doses as low as 3.9 mM. and consisted of a slight rise in tone with an attending decrease in the amplitude of the contractions. With sodium pyruvate the effects were present with a dosage of 2.7 mM. and consisted, as already described by Le Heux (8), of an initial inhibition followed by increases in tone and amplitude.

Activity in Glucose-Free Solutions

The behavior of the isolated gut in glucose-free solutions was compared with that in glucose-containing solutions in a variety of ways. In a great many experiments the gut was allowed to contract in glucose-free solutions until virtual quiescence had been reached. The tracings made by such preparation were

then compared with others contracting in glucose-containing solutions. In one experiment two immediately adjacent portions of the intestine were taken immediately after the death of the rabbit and placed in separate baths. Their contractions were recorded one below the other on the same kymograph. Through one bath was passed a glucose-free solution, through the other, the same standard solution containing 5.5 mM. of glucose. This experiment confirmed on strictly comparable preparations the findings of the previous series of experiments. In still other experiments the behavior of the gut was compared by circumfusing one gut alternately with the two solutions. Finally, the effects of glucose were tested by adding it in isosmotic solution directly to the bath in which an intestinal strip was being circumfused by a glucose-free solution. The results obtained by all these methods correlated very nicely.

Gut preparations used immediately after the death of the animal behave as follows in glucose-free physiological salt solutions: During the first fifteen minutes the behavior is identical to that described for similar guts in glucose-containing solutions. The contractions, which are at first large and even, become steadily smaller and finally irregular. The tone falls steadily. As the second or irregular phase is entered upon there may be a transient rise in tone, but usually the tone falls steadily throughout the experiment. As compared with similar preparations in glucose-containing solutions the irregular period is much shorter. In the final phase the contractions decrease steadily and the tone continues to fall, so that at the end of two or three hours the gut is almost completely relaxed and is contracting only very feebly.

Gut strips which have been stored in the refrigerator previous to use may or may not, depending upon the length of time that they have been stored, have the intermediate irregular period. In its absence the behavior is as follows: During the first ten or fifteen minutes the amplitude of the contractions increases steadily and reaches a maximum. Tone changes during this first period are slight and inconstant. The maximum amplitude which is reached in about fifteen minutes, is gradually followed by a decrease in the contractions and a simultaneous fall in tone. At the end of two or three hours the gut is nearly quiescent.

It was interesting to compare the behavior of the two strips of gut from immediately adjacent portions of the intestine when they were placed in the same glucose-free bath. Both had been stored for eleven hours in the refrigerator, one in a glucose-free physiological solution, the other in a similar solution containing 5.5 mM. of glucose. Although both preparations were

the same length, the contractions of the gut which had been stored in the glucose-containing solution were definitely larger and its tone did not fall as sharply. After two and three-quarters hours had elapsed, the greater relaxation of the gut which had been stored in the glucose-free solution gave it the appearance of having the greater length, and yet its contractions were less than half the size of those of the companion strip.

When a gut was circumfused alternately with a glucose-free, and a glucose-containing physiological solution the following results were obtained: If an unstored preparation was used, as much as one hour might elapse before different behaviors in the two solutions could be detected. In contrast to this, a gut which had been stored for five hours in the refrigerator in a glucose-free solution, showed a different behavior in the two solutions as early as eleven minutes after the start of the experiment. During the irregular phase the only effect that could be observed upon changing the circumfusing fluid to a glucose-containing one was an increase in tone; the irregularity persisted and obscured any effects on amplitude that might have been elicited. When the contractions of the gut became more regular an increase in amplitude as well as tone could usually be observed upon changing to a glucose solution. These effects were often attended by a decrease in the regularity of the gut. As the experiment progressed the response of the gut to the glucose-containing solution became greater and greater. This is presumably due to the fact that the gut is constantly using up its own store of energy and is becoming more and more dependent upon the glucose in the solution to support its activity. This store of energy is used up even in the presence of glucose as is shown by the rapid response of a gut which has been contracting since the start of the experiment in a glucose-containing solution, when this solution is replaced by a glucose-free one. As the gut becomes moribund in the absence of glucose the response to that carbohydrate finally begins to decrease.

When an isosmotic glucose solution is added directly from a pipette to the bath in which the gut is being circumfused by a glucose-free solution, the results obtained are quite in accord with the above findings. At the first part of every experiment there is a period when the glucose evokes no response. With fresh preparations this period may last as long as thirty or fifty minutes. In the case of stored preparations, however, responses may be obtained much earlier. The earliest response was obtained four minutes after the start of the experiment. As the experiment progresses, the responses become greater and greater, provided no glucose is given in the meantime. For this

reason it is important in assay work to maintain the strictest uniformity in the time that elapses between successive doses of carbohydrates. Eventually, of course, the gut reaches a period in which the response to glucose is diminished. In the early part of an experiment, however, it is usually possible to predict upon examining the tracing whether or not a gut will respond to glucose and to some degree even how sensitive it will be. A steady loss of tone and amplitude may be taken as indicating a lack of available energy, and the greater the lack, the greater will be the response to glucose, or, expressed in another form, the more dilute will be the concentration of glucose to which the gut will respond.

With the aid of a rapidly moving kymograph (10 cm. per minute) it is possible to study in considerable detail the immediate effects of glucose as administered directly to the bath. These effects vary qualitatively as well as quantitatively with the condition of the gut and the amount of glucose given. In the early part of an experiment when the gut still has most of its own source of energy, and in those experiments in which a gut, the contractions of which have long been supported by glucose, has only recently been deprived of it, the only effect seen is one of "stimulation." This appears thirty to forty seconds after the beginning of the addition of the glucose to the bath, a procedure which consumes ten seconds. The "stimulation" consists chiefly of an increase in the amplitude of the contractions together with a slight rise in the tone of the gut. There is no conspicuous change in the frequency of the contractions. The contractions increase steadily and reach a maximum in a little more than a minute, maintain this for perhaps two minutes, and then as the glucose is carried out of the bath by the circumfusing solution the contractions decrease steadily until the previous normal is reached. After this the decrease in tone and amplitude is much more gradual.

In the case of guts which have undergone prolonged glucose deprivation, and consequently are highly sensitive to glucose, results similar to those just described can be obtained only if much less glucose (on the order of one per cent of the amount in the previous experiment) is used. If larger doses are used the stimulation is preceded in the majority of the cases by a striking inhibition. The chief characteristic of the inhibition is a decrease in amplitude, which may be nearly complete for the duration of one contraction. The bottom of the curve falls only slightly below the previous normal. The frequency of the contractions is slightly increased. The first sign of inhibition appears about fifteen seconds after the beginning of the addition of the

glucose. It lasts for about twenty-five seconds, and consequently the first contraction that surpasses the previous normal appears, as before, thirty to forty seconds after the beginning of the addition of the glucose. This sequence is illustrated in Figure 2.

The occurrence of this inhibition is not mentioned by Neukirch and Rona (5, 6, 7), but there are some slight indications of it in the tracings published by them. The inhibition is not an artefact caused by a lowering of temperature or by a dilution of active ions in the bath with a nonelectrolyte, as was shown by its absence when an inactive carbohydrate is substituted for glucose. An element of suddenness seemed to be necessary for the production of the inhibition since the inhibition was usually absent in those experiments where the glucose concentration was built up gradually by changing the circumfusing solution from a glucose-free, to a glucose-containing one.

Frequently when a gut long deprived of glucose had developed a considerable sensitivity to that substance, tests were made to see how minute the quantities of glucose were to which it was capable of responding. For these experiments it was found desirable to dilute the 0.318 M. glucose 1 - 200 with isotonic saline in order to render the measuring of such small amounts of glucose more accurate. The smallest amount of this solution to which I have observed a gut respond is 0.09 cc. This is equivalent to 0.026 mgm. of glucose or a concentration of 0.0096 mM. in the 15 cc. bath. Suitable controls with saline alone or with an inactive carbohydrate produced no response.

In view of the important role which phosphates are assuming in carbohydrate metabolism it was thought desirable to determine if the presence of the phosphate ion was essential for the typical glucose response of the gut. For this purpose two glucose-free solutions were prepared. One of them contained all of the constituents listed in Table II; in the other the phosphates were omitted and an equal osmotic equivalent of sodium chloride substituted in their place. The gut was then allowed to contract while being circumfused alternately with the two solutions. At fifteen minute intervals glucose was administered in an amount that would produce a concentration of 4.3 mM. The response obtained in the two solutions was identical.

Many workers have shown an interest in the relative activities of alpha-, and beta-glucose in biological systems. One of the chief hindrances to such studies has been the rapid interconversion of the two isomers in comparison to the time required to elicit the response. Moschini (11), for example, studied the relative amounts of various carbohydrates necessary to maintain a normal blood sugar in rabbits that had received large amounts of

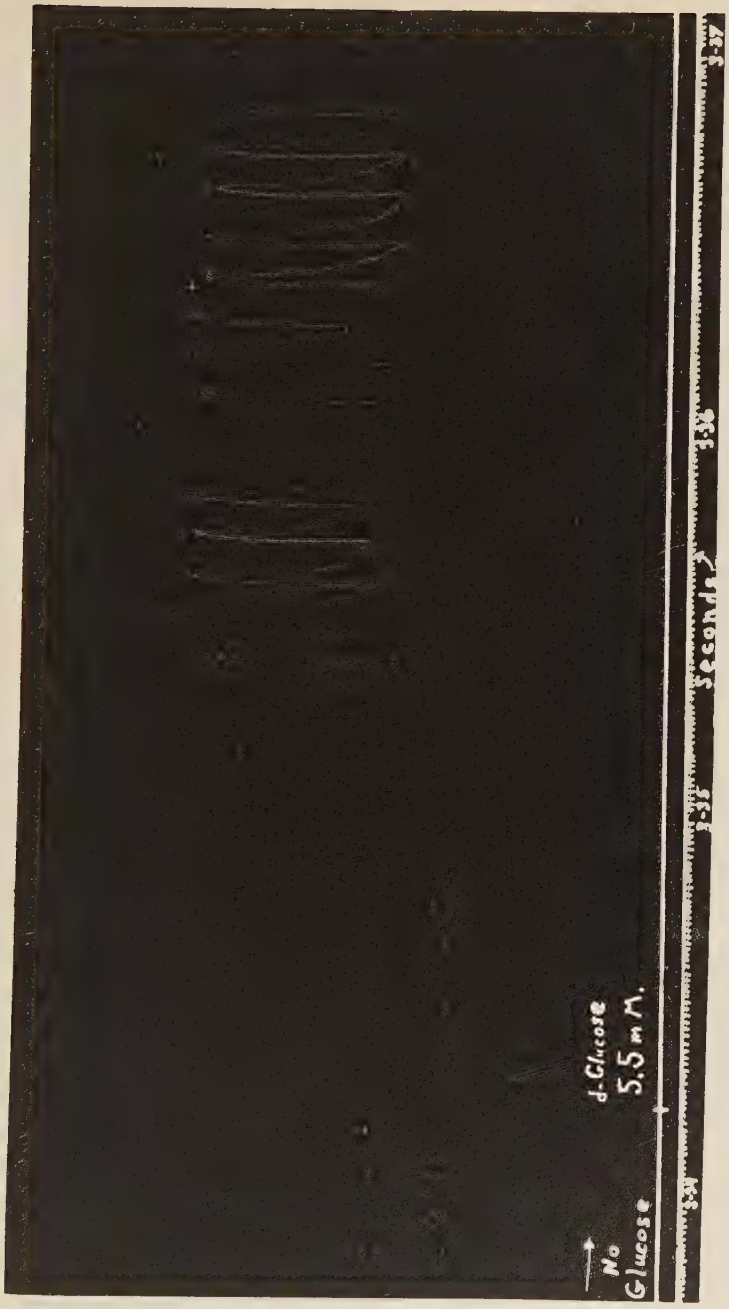

Fig. 2.- Response of a highly sensitive gut to 5.5 mM. of glucose as recorded on a rapidly moving kymograph.

insulin. In comparing alpha-, and beta-glucose he felt that the former was slightly more effective, but he admitted that equilibrium was reached so rapidly that a final statement was not justified.

The isolated gut offers two distinct advantages for studies in this field: In the first place, one is dealing directly with the tissue that is utilizing the sugar, - there is no possibility of an intermediate action by some remote organ to confuse the picture. Secondly, the response of the gut is so prompt that errors due to mutarotation are reduced to a minimum.

The assay was carried out in the following manner: Into each member of a series of 10 cc. volumetric flasks was weighed 0.0573 gm. of alpha-glucose, and into each member of a second series was weighed the same amount of beta-glucose. This quantity of glucose, when dissolved in 10 cc. of 0.81 per cent NaCl, gives a solution that is isosmotic with that used in the bath, and yet one which contains little enough glucose so that even with a sensitive gut submaximal responses are obtained from dosages contained in a convenient volume (0.15 - 0.30 cc.). The contents of one of the flasks was dissolved and a carefully timed and graduated series of dosages was administered to the bath in which a gut had been contracting for a suitable period of time while being circumfused at a constant rate with a glucose-free solution. The purpose of this preliminary study was to determine how small the differences in glucose dosage which the gut could distinguish were, and also to discover what interval had to elapse between successive glucose administrations to permit the gut to return to normal. After the gut had been calibrated in this manner, a comparison of the two sugars was carried out. Alternate doses of alpha-, and beta-glucose were administered in a carefully timed sequence. The contents of each flask was dissolved in 0.81 per cent NaCl at 4° C. immediately before use, and for each administration the contents of a fresh flask was dissolved. Polarimetric studies showed that in the minute and a half that elapsed between the time the saline was added to the glucose and the time the dissolved glucose was added to the bath, not more than two per cent of the alpha (or beta) form had been converted into its isomer. At the higher temperature of the bath the speed of mutarotation was much more rapid. Studies with a polarimeter on glucose dissolved in the bath solution and kept at 38° C. showed that in the minute which elapsed between the addition of the glucose solution to the bath and the maximum response by the gut, an additional eight per cent of the alpha (or beta) form had been converted into its isomer. Therefore at the time of the gut's maximum response, which was used as the criterion of the relative activity of the

two isomers, ninety per cent of the glucose added was still in its original form.

On a gut which could distinguish differences as small as seventeen per cent in glucose dosage, no difference could be shown between the activity of alpha-, and beta-glucose. After making the proper corrections for mutarotation and for the sensitivity of the gut it can be said that these two forms of glucose appear to have the same activity on the isolated gut, or that if there is a difference, the weaker one has an activity of at least seventy-nine per cent of the stronger.

Effect of Substances Other Than Glucose

The first of these substances investigated was d-mannose. Neukirch and Rona (5) report mannose as producing effects equal to that of glucose when administered in concentrations of 0.1 per cent. They state, however, that slightly less mannose than glucose is removed by the gut from the bath solution.

The effects of mannose bear a very close resemblance to those of glucose. In many instances where the two carbohydrates were compared by adding them alternately in isosmotic solution directly to the bath in which a gut was being circumfused with a carbohydrate-free solution, it was impossible to distinguish between the two even upon the closest examination of the tracing. When the conditions are appropriate for a preliminary inhibition to glucose, it can also be produced with mannose. In some cases, however, especially where the gut is hypodynamic, as toward the end of an experiment, it can be clearly seen that the gut is responding more rapidly to glucose than to mannose, even though the two are added in concentrations which produce the same effects.

The mannose used had a specific rotation of 13.87° when examined with a sodium light in a concentration of ten per cent and at a temperature of 20° C. Repeated assays of this sample showed it to have an activity that was approximately sixty per cent of that of glucose. There was some variation from this value with different guts, and in one case the value rose slightly during the course of the assay. A tracing of a typical mannose assay is shown in Figure 3. Note the large dose of glucose given at 1:58 to make certain that the previous responses have been submaximal.

Experiments in which the gut was alternately circumfused with glucose- and mannose-containing solutions showed that even though mannose was capable of supporting the tone of the gut and maintaining the amplitude of the contractions over long periods

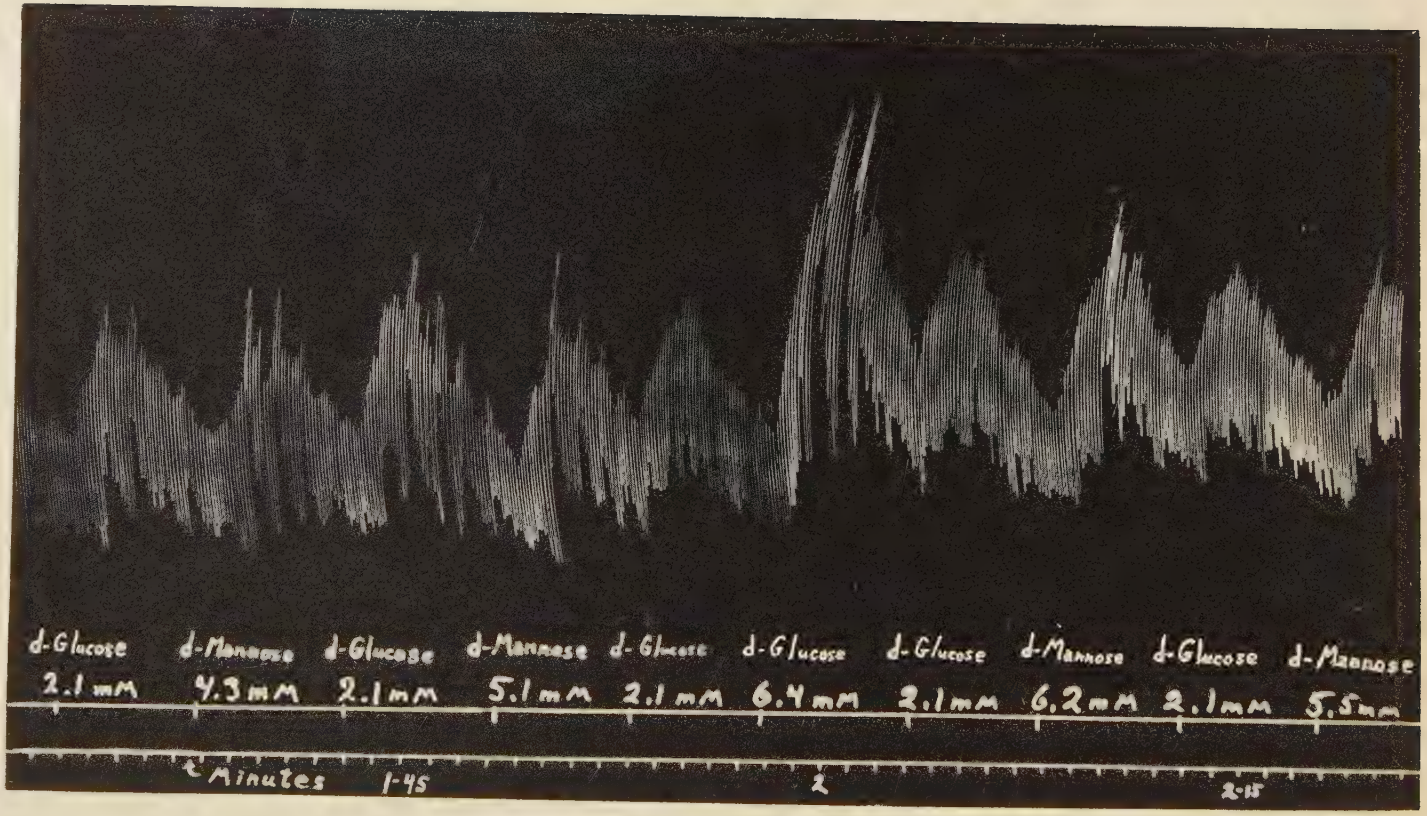

Fig. 3.- An assay of mannose with respect to glucose

of time, higher concentrations of mannose were needed than were used in the case of glucose. With 8.3 mM. mannose the tone was slightly lower than with 5.5 mM. glucose. The behavior in 6.0 mM. mannose was almost equal to that in 3.5 mM. glucose. Thus with more prolonged exposures to the two sugars than occurs during the assays the activity of mannose is also approximately sixty per cent of that of glucose.

The next carbohydrate to be considered was d-galactose. Neukirch and Rona (5) using galactose in a concentration of 0.1 per cent (5.5 mM) found it to be almost devoid of action on the gut, and were surprised that small but significant amounts of it were removed from the bath solution by the gut. In a later paper (7) they report that in higher concentrations galactose does have an action similar to that of glucose. A galactose concentration of 0.5 - 0.6 per cent maintained an activity in the gut similar to that of a 0.05 - 0.01 per cent concentration of glucose. In other words, from their figures, galactose had an activity of approximately fifteen per cent that of glucose.

The first sample of galactose which I assayed was obtained from the Pfanstiehl Chemical Company and classified by them as "Pure." It had a specific rotation of 78.13° when examined with a sodium light in an eight per cent solution and at a temperature of 20° C. In concentrations around 10 mM. it showed an activity that was approximately 2.5 per cent that of glucose. For example, a concentration of 10.7 mM. of galactose in the bath gave approximately the same response as a glucose concentration of 0.26 mM. There was a difference in the responses obtained from the two substances in that the preliminary inhibition was more marked with galactose.

If one assumes that the entire activity of this sample is due to glucose impurity, this would require that it have a specific rotation of 79.7° at the temperature and conditions previously mentioned. The label indicates the presence of one per cent ash. Doubtless other inactive impurities are present.

The second sample of galactose was one labeled "C.P." by Pfanstiehl. It had a specific rotation of 79.40° when examined in a concentration of eight per cent with a sodium light and at a temperature of 20° C. When assayed on the gut it gave the following results: At a concentration of 10 mM. it produced no effect at all, while 0.21 mM. of glucose produced a characteristic response. When the galactose was given in a dose of 32 mM. a response was obtained which superficially resembled that obtained with 0.091 mM. of glucose. According to this the activity of this sample would be 0.28 per cent of that of glucose.

That even this small value does not represent the true activity of galactose is apparent from the following experiment: After a gut has become stabilized it will respond to an evenly spaced series of equal glucose doses with a beautiful uniformity. Let, however, a saline control be given in place of a few glucose doses, or let the doses be omitted altogether for two or three intervals, and now when the glucose is again given (in the same dose as before) the response will be much greater. The explanation of this is the gradually increasing sensitivity to glucose which guts display when they contract in a glucose-free solution. It was found possible to reproduce this phenomenon by interspersing three successive doses of galactose in place of the corresponding doses of glucose in a series of carefully timed glucose administrations. Superficially the galactose was producing a response very much like that of the glucose in place of which it was being given, but the first dose of glucose which came after the galactose, gave the typical exaggerated response, indicating that the galactose had not been exerting a nutritional effect on the gut.

It seems likely that the true action of galactose is one of inhibition and that the rise in tone which follows is nothing more than a recovery-beyond-normal similar to that which is frequently seen to follow the epinephrine inhibition.

The next compound to be investigated was d-fructose. The specific rotation of the sample used was 90.26° with a sodium light in a concentration of eleven per cent and at a temperature of 20° C. When assayed on the gut this sample showed an activity that lay between 0.8 and 0.9 per cent of that of glucose. If all of this activity is due to a contamination of the sample with glucose, it would require that it have a specific rotation of 90.77° under these same conditions.

When the experiment described under galactose is performed with fructose it is not followed by the exaggerated response to glucose, indicating that the fructose is acting in a manner similar to glucose. This action may be due either to glucose impurity or to a weak action of the fructose itself.

It is interesting to note at this point the report of Bollman and Mann (12) that in hepatectomized dogs the presence of the gastro-intestinal tract was essential for the conversion of intravenously injected fructose into glucose. The failure of the isolated rabbit gut to utilize fructose to any great extent may be due either to the asphyxiated condition of its mucosa, or to species variation.

The remainder of the carbohydrates studied were found to be virtually inactive on the isolated rabbit gut. Their activities,

together with those of mannose, fructose, galactose, sodium pyruvate, sodium l-lactate, and sodium d-lactate, are given in Table III. It is important to note that with the exception of the six last mentioned compounds, the values given in the table are maximal, i.e., the true values are smaller. With one compound, dulcitol, the relative insolubility was the limiting factor.

TABLE III

Substance	Activity in Percentage of Glucose
Mannose	60.
Fructose	0.85
Galactose	0.28
Dulcitol	1.5
Sorbitol	0.4
Trehalose	0.1
Xylose	0.1
d-Arabinose	0.095
Sucrose	0.095
Lactose	0.085
Sodium d-Lactate	30.
Sodium l-Lactate	15.
Sodium Pyruvate	50. - 2000.

In the case of the other compounds the limiting factor was the sensitivity of the gut to glucose at the time the compound was assayed. Many of these compounds when given in doses over 40 mM. tend to disturb the tracing. If the gut is able to respond to very small amounts of glucose at the time the substance under consideration is assayed, a value on the order of 0.1 per cent will be obtained for the assay. On the other hand, if larger amounts of glucose have to be given in order to get a response, the apparent assay value will be high. Since it was necessary to replace over twelve per cent of the bath fluid with the carbohydrate solution in order to attain a concentration of 40 mM., concentrations as high as this have a dubious significance, and were never employed.

The last three members of Table III are discussed separately because their activity differs considerably from that of the carbohydrates.

The first of these substances was prepared from commercial lactic acid by the method of Purdie and Walker (13). When sodium

d-lactate is added to the bath in which a gut is being circumfused by a glucose-free solution the effects are quite different from those obtained when glucose is added. First of all, there is no preliminary inhibition in the case of the lactate regardless of the dose in which it is given. When sodium d-lactate is given in doses that produce the same total effect as glucose, it is seen that the gut's tone and the amplitude of its contractions increase much more slowly and last much longer than with glucose. For this reason it is difficult to compare the activity of the two compounds because much of the sodium d-lactate is washed out of the bath before the maximum effects are produced. As found, however, the activity of sodium d-lactate was approximately thirty per cent of that of glucose when the compounds are compared on a molecular basis.

Rona and Neukirch (6) report sodium l-lactate as having either no activity or a very weak one. When this compound was compared with sodium d-lactate by the present assay method it was found to have an activity of approximately fifty per cent of the latter. In other words, the activity of sodium l-lactate is, within the limits of accuracy of the method, entirely due to its sodium d-lactate content.

When sodium d-lactate is compared with glucose over longer periods by the method of alternate circumfusion it is found that the activity of the lactate is somewhat higher than that found by the assay method. A concentration of 10 mM. of sodium d-lactate produces about the same effects as 5.5 mM. of glucose. Some differences are noted, however. On prolonged exposure to the lactate solution there is much more tendency to spasm of the gut, that is, the amplitude of the contractions is decreased by virtue of a diminished relaxation. At higher concentrations of sodium d-lactate this tendency is even more conspicuous. When glucose is added to the bath in which a gut is contracting in the presence of lactate, it produces this same spasm. There is evidence of synergism between glucose and sodium d-lactate in the production of the spasm, for when the gut is circumfused alternately with concentrations of the two substances that produce the same effects, a momentary spasm is produced each time a change is made from one solution to the other.

In the case of sodium pyruvate widely divergent results were obtained when its activity was assayed against that of glucose on the isolated rabbit gut. Le Heux (8), as previously mentioned, accounted for the activity of sodium pyruvate on the assumption that it esterified with the choline which is normally present in the intestinal wall and formed the more active pyruvyl choline. He noted that after repeated washings the response of

the gut to sodium pyruvate was diminished, and he accounted for this on the assumption that the choline had been washed out. As shown by Jendrassik and Tangl (9) these assumptions are false, for while atropine prevents the action of choline esters it does not prevent the action of sodium pyruvate. In my assays sodium pyruvate prepared from one sample of pyruvic acid showed activities that ranged from twenty times that of glucose to less than fifty per cent that of glucose. During one assay the value fell from over 150 per cent at the start to less than fifty per cent at the end of the experiment. On one atropinized gut sodium pyruvate showed an activity which was thirteen times that of glucose. That this high value was not due to atropinization was shown on another gut where the activity before and after atropinization was about three and one-half times that of glucose. In general the higher assay values were obtained at the early part of experiments and the low values toward the end when the gut was hypodynamic and extremely sensitive to glucose. If one recalls the statement made in the earlier part of this paper that sodium pyruvate is able to stimulate the gut even in the presence of an adequate supply of glucose, the explanation for these widely divergent results is evident. As a gut is deprived of glucose its sensitivity to that substance increases. The sensitivity to sodium pyruvate does not increase correspondingly and consequently its assay value falls. That the action of sodium pyruvate is nutritional as well as stimulating is shown by the fact that in concentrations of approximately 1 mM. it is able to support the contractions of the gut over long periods of time. In concentrations higher than this there is a tendency to spasm similar to that observed with sodium lactate.

SUMMARY

1. Methods are described for the quantitative comparison of the activities of carbohydrates on the isolated rabbit gut, both as regards their brief and prolonged effects.

2. The behavior of the gut in glucose-free solutions and solutions containing various concentrations of glucose are described and compared.

3. The activity of alpha-, and beta-glucose was found to be identical.

4. Mannose has an activity which amounts to sixty per cent of that of glucose.

5. The activity of other carbohydrates is insignificant and is probably referable to glucose impurities.

6. Sodium d-lactate has an activity that is thirty per cent of that of glucose. Qualitative differences in its behavior are also described.

7. Sodium l-lactate shows an activity that is proportional to its d-lactate content.

8. Sodium pyruvate has a very erratic action. Its activity varies from twenty times that of glucose to fifty per cent.

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